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Biological Sampling in Support of the Fisheries Act

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BIOLOGICAL SAMPLING IN SUPPORT OF THE FISHERIES ACT

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SUMMARY

The purpose of this document is to review biological sampling methods used during investigations of alleged spills of deleterious substances into waters frequented by fish. The first part of the document traces the development of the Fisheries Act up to the current amendments and regulations. Related provincial acts, particularly the Alberta Clean Water Act and the Agricultural Chemicals Act, are also discussed.

The second part of the report initially reviews the cause of natural fish kills and the concept of a "mixing zone", in which surface water quality standards may be exceeded under authority of the Fisheries Act or related provincial acts. The report then describes biological and chemical sampling methods particularly in support of:

i. collection of water and effluents for laboratory bioassays;

ii. pathological examination of fish tissues;

iii. assessment of off-flavours in fish;

iv. chemical analysis of water and fish.

1.0 INTRODUCTION

1.1 Objective of Report

The objective of this report is to review the biological sampling methods used during investigations of alleged spills of deleterious substances into water frequented by fish. The first part of the report traces the development of the Fisheries Act up to and including current regulations and amendments. This material is designed to give the reader appropriate background information, with case examples, for actual on-site investigations.

The second part of the report describes biological sampling techniques at the site of an alleged spill. Consideration is given to photography, collection of fish and water for chemical analysis, collection of fish for histopathologic evaluation, tainting effects and acute and chronic effects in fish.

1.2 History of the Fisheries Act

The Fisheries Act was originally promulgated by the Government of Canada as The Fishery Acts of 1868. As such, it is one of Canada's earliest pieces of environmental legislation, specifically referring to the term "pollution". The Fishery Acts (1868) prohibited the deposit of "deleterious substances" into any water where fishing was carried on. The definitions of deleterious substance included "poisonous matter" such as "lime, chemical substances or drugs",

"sawdust" and "mill rubbish". The Minister could exempt any stream or portion of a stream, from the provisions of The Fishery Acts if he considered its enforcement was not requisite for the public interest. Maximum fines provided by the Fishery Acts were not to exceed $100 or imprisonment for not more than two months. The Fishery Acts were amended in 1873 and renamed The Fisheries Act. Further amendments in 1894 contained specific provisions to control discharges from saw mills. The amendments provided a fine of $20 plus costs for the first offence, $40 plus costs for the second offence plus an additional penalty of up to $10 for every day during which the offence continued, and up to $100 plus costs for the third and subsequent offences. The amendments of 1895, 1897, and 1898 continued to emphasize control over saw mills and, in particular, the deposit of slack, debris and sawdust into rivers. Further changes were made in 1906, and in 1927 provision for the development of regulations was made. Two of the specific objectives of the regulations were:

i. "for the better management and regulation of the sea coast and inland fisheries";

ii. "to prevent or remedy the obstruction and pollution of streams".

Additional relatively minor amendments were made in 1906, 1927, and 1932. There was an office consolidation of all of the previous changes in 1939 and again in 1948. The 1961 version provided for fines of not less than $100 and not more than $1000 for the first offence, or imprisonment for a term of not less than one month and not

more than six months, or both. For a second and each subsequent offence, the fine was not less than $300 and not more than $2000, or imprisonment for a term of not less than two months and not more than twelve months, or both.

1.3 Current Amendments

The Fisheries Act, RSC 1970 S.32(2), makes it an offence for any person, including the Crown, to deposit or permit the deposit of a "deleterious substance" of any type in any water frequented by fish. Oil, coal, ashes, pesticides, toxic industrial effluents or other prejudicial or harmful substances may not be deposited in any water containing fish nor may the remains or offal of fish or mammals be deposited upon or along shorelines.

The Fisheries Act (S.31 (1)) also makes it an offence for any person to carry on any work or undertaking that results in the harmful alteration, disruption or destruction of fish habitat, unless authorized by the Minister or under regulations. The Act also prohibits any person from destroying fish by any means other than fishing. Maximum fines for offences under The Fisheries Act are $50,000 for the first offence and $100,000 for the second and third offences.

Under Section 33(2), a "deleterious substance" is:

i. "any substance that, if added to any water, would degrade or alter or form part of a process of degradation or alteration of the quality of that water

so that it is rendered or is likely to be rendered deleterious to fish or fish habitat or to the use by man of fish that frequent that water, or

ii. any water that contains a substance in such quantity or concentration, or that has been so treated, processed or changed, by heat or other means, from a natural state that it would, if added to any other water, degrade or alter or form part of a process of degradation or alteration of the quality of that water so that it is rendered or is likely to be rendered deleterious to fish or fish habitat or to the use by man of fish that frequent that water."

The federal Cabinet may by regulation prescribe as being deleterious:

i. a specific substance or class of substances;

ii. concentrations or quantities of substances or classes of substances in water; or

iii. any substance subjected to treatment, process or change.

If a substance causes or is likely to cause sublethal effects to fish at a specific concentration, then it is a deleterious substance (Regina v. Suncor Inc., 1983).

Deposit "means any discharging, spraying, releasing, spilling, leaking, seeping, pouring, emitting, emptying, throwing, dumping or placing". A deposit is considered to have taken place whether or not any act or omissions resulting in the deposit is intentional.

Water frequented by fish means "Canadian fisheries waters". One court case (Regina v. Cyanamid Canada Inc., 1981) held that it is not material or relevant whether a particular waterway is fished commercially; the only important factor was that the waterway contained fish. Another court (Regina v. MacMillan Bloedel [Alberni] Ltd., 1982) did not follow that approach, noting that the stream in question was not a fishery or part of one to be identified as a fishery; the stream contained no fish of commercial or sporting value, and did not form part of the habitat of anadromous fish. Another court found that fish do not have to occupy the water continually or even frequently.

Proof that the water in question is frequented by fish must be made by the Crown beyond a reasonable doubt. Proof may include angling and commercial fishing records, scientific reports, and results of test netting. Fish include shellfish, crustaceans, marine-animals, plus the eggs, spawn, spat and juvenile stages of these species.

Once a substance has been found to be deleterious when added to water, then it becomes an offence to deposit it into water in any other location (Regina v. MacMillan Bloedel [Alberni] Ltd., 1982). Substances held to be deleterious include:

i. diesel fuel (Regina v. Placer Devs. Ltd., 1983);

ii. bunker C oil (Regina v. MacMillan Bloedel [Alberni] Ltd., 1982);

iii. ammonia (Regina v. Cyanamid Canada Inc., 1981);

iv. sewage (Regina v. District of North Vancouver, 1982);

v. pentachlorophenol/tetrachlorophenol wood preservatives (Regina v. Western Stevedoring Co.,1982);

vi. sediments, silt, clay, sand and gravel (Regina v. Stearns-Roger Engineering Co., 1973; Regina v. Pioneer Timber Co., 1979);

vii. log conditioning water (Regina v. MacMillan Bloedel Industries, 1976).

1.4 Sublethal Effects

Most of the cases under Section 33(2) have dealt either directly or indirectly with acute toxic effects as an interpretation of "deleterious". In Regina v. Coulson Prescott Logging Ltd. (1982), however, it was held that logging debris and silt, while not deleterious by themselves, may become deleterious over a period of time. In Regina v. Suncor Inc. (1983) it was held that a concentration of oil and grease of greater than 10 mg/L (10 parts per million) in water could induce sublethal toxic effects in fish; hence such a concentration should be described as "likely to be deleterious". The judge had to rule on four questions:

i. what actually went into the river;

the form of the substance released into the river;

the analysis available to determine the composition of the discharged material;

ii. the toxicity of the material discharged to the river;

iii. the concentration, transport and/or dispersion of the discharged material;

iv. observed effects on aquatic life in the river.

After reviewing the evidence on these points in Regina v. Suncor Inc. (1983), a conviction was registered indicating that the discharged material would likely cause deleterious effects to fish.

A potential sublethal effect is the tainting of fish tissues following an untoward discharge of organoleptic substances. Numerous compounds are known to impart an off-flavour to water and tissues including aldehydes, ketones, acids and phenolics. Off-flavour imparted to fish may constitute a deleterious effect on the "use by man of fish that frequent that water".

1.5 Regulations

Regulations have been developed which provide exemptions from the prohibitions or discharges of deleterious substances. The regulations developed to date are:

i. Chlor-Alkali Liquid Effluent Regulations;

ii. Pulp and Paper Effluent Regulations;

iii. Petroleum Refinery Liquid Effluent Regulations;

iv. Metal Mining Liquid Effluent Regulations;

v. Meat and Poultry Products Plant Liquid Effluent Regulations;

vi. Potato Processing Plant Liquid Effluent Regulations.

The Fisheries Act, S.33(2), allows an exemption to be site- or operation-specific. An example is the Alice Arm Tailing Deposit Regulations, which were developed by Environment Canada and the owner of the Amax Kitsault Mine in British Columbia. The regulations were developed despite the Metal Mining Liquid Effluent Regulations prohibiting the disposal of unconfined solid tailings. The Fisheries Act does not require public notice prior to an exemption being granted.

2.0 RELATED ACTS

The Canada Water Act empowers the federal government to make agreements with the provinces for comprehensive water resource management. Each province then promulgates its own set of Clean Water regulations controlling the discharge of toxic effluents or nutrients into water.

The Alberta Clean Water Act requires operators to report within 24 hours:

i. any uncontrolled release of contaminants, or

ii. any controlled release of contaminants not authorized by a license, or

iii. any accidental spill or discharge.

"Contaminant" means:

i. any solid, liquid, gas, odour or combination of any of them, or

ii. heat in water as a result of the use of water in an industrial plant or municipal plant or discharged by or from an industrial plant or municipal plant.

The Act requires the issuance of a licence to discharge effluents to surface waters in Alberta. The licences generally specify that the discharge should not be deleterious to fish. Under authority of the Act, the following regulations and guidelines have been developed:

i. Waste Water Effluent Guidelines for Alberta Petroleum Refineries;

ii. Clean Water (Industrial Plant) Regulations;

iii. Clean Water (Municipal Plant) Regulations;

iv. Fertilizer Plant Waste Water Effluent Guidelines;

v. Fluoridation Regulations;

vi. Gas Processing Plants - Waste Water Management Standards.

The Agricultural Chemicals Act provides authority for the safe use, handling, storage, application and disposal of registered pesticides. The act specifies that:

a. No person shall apply, deposit, add, emit, discharge or cause or permit the application, deposition, addition, emission or discharge of a pesticide or a substance or thing containing a pesticide

1. into, on or over an open body of water, or

2. within

i. 30 horizontal meters, or

ii. any other distance prescribed in the regulations, of an open body of water.

b. Subsection (1) does not apply to a person who:

1. holds a licence or permit in accordance with the regulations or act to do so, or

2. is exempted by the regulations from the requirement to hold a licence or permit.

A licence may only be issued for registered compounds, which are listed in the Pesticide Sales Use and Handling Regulations. Because registered compounds have undergone an extensive series of tests, it is assumed that deleterious effects to fish and other aquatic organisms are either minimal or non-existent.

3.0 PRELIMINARY CONSIDERATIONS IN SAMPLING

3.1 Natural Mortality

The presence of dead fish does not necessarily mean that a deleterious substance has been deposited into a lake or river. Fish die much more frequently, and in greater numbers, from natural causes than from the deposit of anthropogenic substances. Factors such as spawning, production of toxic algal metabolites, low dissolved oxygen concentrations and elevated concentrations of hydrogen sulfide and ammonia (from natural sources) all contribute to fish mortality.

In Alberta, large-scale fish kills can be observed around the shore of lakes during the early summer. The fish that die typically inhabit shallow water in eutrophic lakes where oxygen concentrations may fluctuate dramatically (supersaturated in the day, low oxygen at night). The problem is widespread in Alberta and affects littoral species such as stickleback and juvenile perch. Extensive natural kills have also been reported in Alberta involving salmonids. Many of these species spawn in shallow water, after a lengthy migration in rivers. Relatively rapid changes in temperature occur in shallow water; in addition, the risk of abrasion to the fish increases.

3.2 Mixing Zones

A mixing zone, located immediately downstream of an industrial or municipal discharge point, is an area of noncompliance with surface water quality standards, permitted under authority of the Fisheries Act or related provincial acts. Although fish kills are seldom reported within the mixing zone, discharges may be toxic to fish, at least under laboratory conditions. In addition, there is scope for bioaccumulation of toxic and/or tainting substances within the mixing zone.

The design of waste treatment facilities may be based on Best Available Technology (BAT), Best Practicable Technology (BPT), Best Conventional Technology (BCT), plus a number of other control scenarios. Because of economic and technical matters, it may be costly but possible to implement BAT. When an effluent is toxic to

fish, using an alternate process such as BPT, a compliance schedule may be developed, requiring a gradual improvement in effluent quality. The timetable for effluent improvement, developed under authority of the Clean Water Act, may extend over the course of several years. Hence, no violation of the Fisheries Act (33(2)) occurs under these conditions.

4.0 GENERAL PRINCIPLES FOR SAMPLING

4.1 Techniques

The strategy outlined in the following sections involves the principles of photography, chemical analysis, histopathology, aquatic toxicology, and production of off-flavours. It must be emphasized here that all such techniques and investigations should follow a strict protocol, and that laboratory evaluation cannot compensate for inadequate sampling.

4.2 Sample Identification and Background Information

All samples and other material associated with an investigation must be identified by:

i. date;

ii. time;

iii. location;

iv. name, address and telephone number of person taking sample.

A detailed written description of all samples, circumstances and contact persons should be made as soon as possible.

All fish collected should be individually logged according to species, length and weight. Each fish (or group of fish if very small) should be identified by a numerical code, which is also recorded in the log book.

4.3 Photography

Photographs provide a record of what the investigator saw at the time of the incident. Photographs should include the general area, source of discharge, apparent affects of the discharge on water quality and/or sediment quality, and dead or dying fish. If the discharge included physical debris such as slash or gravel, these should be photographed. Other items that should be photographed are equipment and facilities involved in the alleged spill as well as sampling sites and equipment.

If possible, photographs should include some object which can unequivocally prove that the photograph is of a particular site. Photographs should be signed and dated by the photographer at the earliest opportunity and a log or record of photographs taken should be made.

4.4 Characterization of Water and Effluent

Watercourse and effluent characteristics to be determined are:

i. pH;

ii. total oxygen demand;

iii. chemical oxygen demand;

iv. total dissolved solids;

v. suspended solids;

vi. dissolved oxygen;

vii. total organic carbon;

viii. conductivity;

ix. temperature.

These determinations should be made on site, if possible.

5.0 SAMPLING STRATEGY AND COLLECTIONS

Once the preceding points have been considered, investigations should initially identify the type of deleterious substance, its origin, and the nature of the fisheries resource. Even if a substance has already been established in case law to be deleterious, samples should be taken to add to the data base concerning the incident.

Sampling strategies should consider acute and chronic effects in fish whenever possible. Acute effects are those in which the exposure is for less than 90 days and may lead to the outright death of fish over a relatively short time period, ranging from a few minutes to

less than 90 days. Chronic effects develop after exposure to a pollutant for at least 90 days, and may not lead to immediate mortality. Chronic effects may apply to individual fish or to populations. The latter category causes the greatest concern in environmental cases because of the potential long-term impact on fisheries. Examples of chronic effects on populations include reduction in fecundity and siltation of gravel (where some species spawn).

5.1 Acute Toxic Effects

Histopathologic Evaluation. Small fish should be fixed in neutral buffered saline formaline (one part fish/nine parts fixative). The fixative is prepared as outlined in Appendix A. Larger fish (>10 cm in length) should be slit open at the abdomen and placed in fixative. The samples do not need to be refrigerated and can remain in the fixative until further processing in the laboratory.

Bouin's solution is well suited as a laboratory fixative, and may also be used in the field (Table 1 and Appendix A). One of the drawbacks of Bouin's solution is that the fixed tissues must be transferred to formalin within 24 h of preservation. Such a transfer may not be practical during the course of an ongoing spill.

Dead fish may be suitable for chemical analysis. However, since tissues break down rapidly upon death, the samples will generally be of little use for pathological analysis. A standard operating

Table 1. Guidelines for the Sampling of Fish

On Site

- Photograph (35mm, colour) dead/dying fish, and general area including discharge points if identifiable.
- Collect and freeze dying and dead fish for chemical analysis.
- Preserve dying fish in neutral buffered saline formalin for pathological evaluation. If Bouin's fixative is used, the solution must be poured off and replaced with 10% buffered formalin within 24 hours.
- Obtain effluent sample for acute bioassay procedure and for chemical analysis (see Table 2 for sample types).

Upstream (Control)

- Net fish for chemical analysis and pathological evaluation.
- Do not collect fish by electroshocking (causes severe alteration to gill structure).
- Collect water samples for bioassay and chemical analysis. Sampling procedure (Table 2) should be similar to the on-site technique.

Downstream*

- Net fish for chemical analysis and pathological evaluation. Do not collect fish by electroshocking (causes severe gill alteration).
- Collect water samples for acute bioassay procedure and chemical analysis. Sampling procedure (Table 2) should be similar to the on-site technique.

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*To establish impacted area; samples may be taken at intervals below the spill. The exact distance varies from spill to spill ranging from <0.05 km to >100 km.

procedure for the preservation and necropsy of fish at the Alberta Environmental Centre is given in Appendix A.

Laboratory Bioassays. Water and effluent samples should be collected for laboratory bioassays. These tests are a means of determining the acute toxicity of substances potentially deleterious to fish. The standard operating procedures used for receipt of samples, acute bioassays and chemical reference tests at the Alberta Environmental Centre are given in Appendices B, C, and D, respectively.

In collecting water and effluent samples for bioassay, consideration should be given to the dispersal of the pollutant in the water column, the movement of the pollutant away from the spill site, and the duration of the spill. The minimum quantity of liquid to be collected is 20 L (5 gallons); a 40 L sample permits the use of multiple dilutions, essential in the calculation of an LC_{50} (concentration lethal to 50% of the fish). If the spill is ongoing, a much larger sample can be collected.

In many instances, it will only be possible to collect a grab sample for bioassay purposes (Table 2). A grab sample is defined as a single sample collected at a particular time and place. However, if the source is known to be fairly constant in composition over a considerable period of time or over substantial distances in all directions, then the sample may be said to represent a longer time period. Longer term spills may require the collection of composite and integrated samples. A composite sample is a mixture of grab samples collected at the same sampling point at different times.

Table 2. Characteristics and Use of Grab, Composite and Integrated Samples

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Grab Sample

Used during a single incident spill over a relatively short time period, generally <1 day. Sampling considers the physico-chemical characteristics of the polluting substance.

Examples:

i. Floating oil and grease may be characterized by a grab sample taken near the surface.

ii. Silt/gravel cannot be adequately sampled using the grab technique because such material distributes through the water column; integrated samples need to be used.

Composite Sample

For a one-day spill, collections are made every hour (or other time interval) at the same location to obtain daily average.
In the case of an ongoing spill, collection of one sample per day.
Intermittent discharges may require on-site modification to composite sampling technique.

Integrated Sample

Used primarily to address the physico-chemical characteristics of a specific pollutant.

Examples:

i. Floating oil and grease require horizontal integration of near-surface samples.

ii. Silt/gravel spills require vertical integration of samples to obtain depth profile.

Integrated samples are mixtures of grab samples collected from different points simuitaneously, or as nearly so as possible.

5.2 Chronic Effects

Chronic effects are those that develop after a lengthy exposure period (>90 days). Laboratory investigations into chronic effects may also be lengthy and require large volumes of replacement water. The sampling protocol used to investigate chronic effects is essentially identical to that outlined earlier with the exception that greater volumes of water need to be collected.

Storage of water and effluent samples may become a problem during chronic studies. Most types of effluents cannot be stored beyond 5 days due to deterioration of the sample; therefore, the sample needs to be transported to the laboratory as soon as possible after collection. Some types of materials, such as silt/gravel, do not deteriorate significantly with time, and storage is not a major problem.

There are a number of different toxicological procedures that might be used to determine the chronic toxicity of an effluent. These include:

i. egg and fry development and survival;

ii. fecundity of fish species with a relatively short reproduction cycle;

iii. gill structure/function studies;

iv. enzyme activity in gills and livers;

v. feeding and growth.

The decision to implement any one of these procedures would depend on the nature of the deleterious substance and the availability of specific fish stocks in the laboratory at the time of the spill and the potential cost of the evaluation.

6.0 TAINTING SUBSTANCES

6.1 Background

Many of the compounds produced by the fossil fuel and pulp and paper industries have the potential to impart off-flavours to water, fish, shellfish and other species. Tainting potentially affects "the use by man of fish that frequent . . . water". Most taints from the fossil fuel sector originate from petroleum and its byproducts discharged during the production and/or refining of oil. Oil spills are also occasionally implicated in tainting.

Petroleum hydrocarbons actually have very little taste, namely sweetness, sourness, bitterness and saltiness. The off-flavour is, in fact, an organoleptic sensation, caused by the volatilization of hydrocarbons. Non-petroleum substances, such as dimethyl sulfide, are periodically implicated in imparting an off-flavour, also through the volatilization route.

Tainting substances can also be produced by algae, actinomycetes and, to a lesser degree, by bacteria. Such taints come from entirely natural sources and should be distinguished from spills and other anthropogenic inputs.

Many hundreds of compounds can taint water and tissues (Tables 3 and 4). Some of the most potent compounds, with an Odour Index of $>10^6$, include: mercaptans, alkenes, sulfides, low molecular weight butyrates, ethers and alkylamines. Those with an Odour Index of 10^4-10^6 include: di- and tri-alkylamines, ethelesters, aldehydes, ethers and alcohols. Examples of less potent compounds (Odour Index $<10^4$) are alkanes, phenolics and lower alcohols. In general, the presence of a double bond enhances the Odour Index, as does some functional groups, especially CHI_3, CH_3SH and $ClC{\equiv}N$.

The time required for sorption of tainting substances is highly variable, ranging from just a few minutes to several days. Butanethiol, administered at 0.1 mg/L can produce an off-flavour in rainbow trout in 15 min (Shumway and Palensky, 1973). Equally low concentrations of 2,4-dichlorophenol can taint the same species in 10-15 min whereas concentrations of 0.01 mg/L produced an off-flavour in 1.27 h. By contrast, Persson (1984) found that when the eels Anguilla anquilla were exposed to chlorophenol concentrations of 0.0001-0.1 mg/L, a taint did not develop for 1-11 days depending on the concentration. In general, purging the off-flavour is a relatively slow process, taking days to weeks; chlorophenol, for

example, required 21 days for reduction to non-detectable levels in several freshwater fish species (Persson, 1984).

In some cases, oysters have been tainted with as little as 0.002 mg/L of dissolved hydrocarbons whereas fish have acquired an offensive odour after being exposed to petroleum at 0.01 mg/L (Connell and Miller, 1981). These concentrations should, however, be taken as

Table 3. Threshold Odour Concentration for Oily Substances in Water.

Compound	Concentration in water (mg/L)	Compound	Concentration in water (mg/L)
Acenaphthene	0.08	Indene	0.001
Benzene	0.07	1-Methylnaphthalene	0.01
Benzothiazole	0.08	Naphthalene	0.005
n-Butylbenzene	0.1	Tetralin	0.01
t-Butylbenzene	0.05	Trimethylbenzene	0.5
Diethylbenzene	0.001	Diesel fuel	0.0005
Cumene	0.1	Fuel oil	0.002
Dibenzofuran	0.003	Naphtha	0.016

Sources: Zoeteman et al. (1971), Alexander et al. (1982), Lillard and Powers (1975).

Table 4. Examples of Chemical Compounds and Mixtures of Compounds Capable of Imparting an Off-flavour to Tissues of Fish and Other Aquatic Species.

Compound	Concentration in water (mg/L)	Compound	Concentration in water (mg/L)
	Chemical Compounds		
Acetophenone	0.5	Naphthol	0.3-1
n-Butanol	10	p-Nitrophenol	10
n-Butylmercaptan	0.06	Phenol	1
p-Butylphenol	0.03	o-Phenylphenol	0.1-1
m,o,p-Chlorophenol	0.02-0.06	Pyridine	5-28
m,o,p-Cresol	0.1-2	Pyrogallol	20-30
Ethylbenzene	0.25-0.5	Toluene	0.25-50
Guaiacol	0.08	2,4,6-Trichlorophenol	<0.05
Naphthalene	1-3.4	Xylenol	1-5
	Mixtures of Compounds		
Aromatic naphtha	0.1	Effluent, oil refinery	0.25
Coal coking waste	0.1	Effluent, petrochemical	3.3
Coal tar waste	0.1	Kerosene	0.1->20
Cutting Oil	>15	Oil, disperser	6.4
		Oil, emulsifiable	15

Sources: Connell and Miller (1981), Persson (1984), Shumway et al. (1973), Verschueren (1977).

generalizations, and it may be quite difficult to predict what concentration of complex material will taint aquatic organisms.

Most tainting substances have an objectionable taste and odour well below the concentration that would induce toxic effects in human consumers. The most frequently reported symptoms following consumption of tainted fish include vomiting, nausea and diarrhea. It is widely assumed that once an off-flavour disappears, the foodstuff is suitable for consumption. Yet many petroleum products (such as polycyclic aromatic hydrocarbons) have a relatively long half life in tissues and are likely to persist well after the taint has disappeared. Although little research has been done on this topic, it is apparent that tainted fisheries should not be immediately reopened without chemical analysis of tissues.

6.2 Sampling

Because tainting can be induced by naturally occurring substances, it is necessary to collect upstream control fish, as well as those downstream of the alleged deposit (Table 5). Fish can be collected using any technique such as gill netting and angling. Electroshocking can be used in collecting fish for tainting studies because the damage caused to gills and other tissues will not affect the flavour of the fish. Fish should be assigned a numeric code, cooled immediately and frozen as soon as possible after collection. Information to be recorded for each fish should include date, time, location, and name of person taking the sample. Since many species of fish are highly

Table 5. Data Required for Investigations of Tainting of Fish.

Principal investigator

Laboratory

Dates of samples, exposure, and taste evaluation

Characteristics of stream, river, lake or effluent plus dilution water:

i. pH
ii. total oxygen demand
iii. chemical oxygen demand
iv. total dissolved solids
v. suspended solids
vi. dissolved oxygen
vii. total organic carbon
viii. conductivity
ix. temperature

Species name, weight, length, number of fish exposed to each concentration, source

Description of exposure system and length of exposure

Effects on fish

Flavour evaluation

i. Number of panel members
ii. Relative experience level of each panel member, for example:
frequent tasting experience
some tasting experience
no previous tasting experience
iii. Summary of raw data

Statistical method used to evaluate data

Source: ASTM (1984).

migratory, particularly in rivers, there may be considerable intermingling of tainted and nontainted stocks. Accordingly, upstream control samples may have to be taken at a number of sites to estimate the frequency of tainted fish. The same generally applies to

downstream exposed fish. Water quality and other data, as listed in Table 5, should also be collected.

An alternate method to evaluate an effluent for flavour impairment is to place caged fish directly in the effluent streams for a period of up to 10 days. The specific technique is described in detail in ASTM (1984). The method is limited to an ongoing spill and needs continuous supervision.

Once the fish are ready for analysis, they should be cleaned and eviscerated. Fish are baked at 190°C for 20-30 min (ASTM, 1984). The palatability of the fish is determined by a taste panel. These panels follow a strict operating procedure (Appel, 1985; ASTM, 1984; Bartels et al., 1986).

7.0 PHYSICAL AND CHEMICAL ANALYSIS IN SUPPORT OF BIOLOGICAL SAMPLING

7.1 Water

The effects of a deleterious substance on fish can be significantly modified by physico-chemical conditions in the receiving waters. The toxicity of a substance may be increased either additively or syngergistically, or be decreased in comparable proportions. Therefore, a number of routine water quality parameters should be measured whenever possible, including temperature, pH, conductivity, suspended solids, dissolved oxygen, and total dissolved

solids. Such measurements provide a background for interpretation of biological data.

It is also often necessary to collect water samples for analysis of heavy metals and specific organic compounds, depending on the nature of the discharge. Such samples may identify the toxic substance and those agents that affect toxicity. The sampling strategy should be similar to that outlined for the collection of bioassay samples.

Sample preservation is highly specific; the reader is requested to follow the information in Table 6 whenever possible for preservation.

Preservatives should be added to the samples immediately. If preservatives are unavailable in the field, they should be added as soon as possible. If impossible to add the preservatives at the time of collection, record the time delay between collection and preservation.

PCB (polychlorinated biphenyls), hydrocarbons (to be used when oil is suspected but not present on the water surface), pesticide, and herbicide samples must be collected in a 1 litre amber glass bottle, kept cool and dark and delivered to the laboratory within 24 h. Samples other than water must be collected in a 500 ml ointment jar, with a teflon liner, that are supplied by the laboratory.

Do not add water to the preservative: preservatives must be added to the sample water.

All samples should be kept cool (4°C is ideal).

Table 6. Sample preservation chart

Parameter Group	Tests Included in Parameter Group	Preservative	Container	Holding Time
Routine	pH, Conductivity, Fluoride, Calcium, TDS, Nitrates, Magnesium, Silica, Nitrites, T-Hardness, Chloride, Total-Alkalinity, Sodium, Sulfate, Bicarbonate, Potassium, Iron, Carbonate	Cool to 4°C and transport to lab as soon as possible.	500 mL polyethylene	7 days
Partial Routine	Fluoride only, Nitrate only, etc.	Cool to 4°C.	125 mL polyethylene	7 days
Metals	Cadmium, Manganese, Arsenic, Copper, Chromium, Selenium, Nickel, Vanadium, Silver, Cobalt, Molybdenum, Aluminum, Zinc, Lead, Beryllium	Blue Dot (5 mL 1:1 HNO_3)	500 mL polyethylene	3 months
Mercury	Mercury	Yellow Dot (2 mL HNO_3, 2 mL $K_2Cr_2O_7$)	125 mL polypropylene	14 days
General-1	BOD, Tannins & Lignins, Odour, Residues, Turbidity, Surfactants, Colour, Hexvalent-Chromium	Cool to 4°C and analyze as soon as possible.	2000 mL glass (with teflon liner)	6 hours
Oils & Greases	Oils & Greases	Black Dot (5 mL 1:1 H_2SO_4)	1000 mL glass (with teflon liner)	24 hours
Phenolics	Phenolics	Black Dot (5 mL 1:1 H_2SO_4)	1000 mL glass (with teflon liner)	24 hours
Cyanide	Cyanide	Red Dot (5 mL 6N NaOH)	1000 mL glass (with teflon liner)	24 hours
General-2	COD, Nitrogen, Total Kjeldahl, Total Phosphorus, Ammonia	Brown Dot (2 mL 5% H_2SO_4)	125 mL polyethylene	7 days
Low-Level Nutrients	Ortho-phosphate, Nitrate nitrogen, Nitrite nitrogen	Cool to 4°C and keep out of sunlight. Transport to lab as soon as possible.	125 mL polyethylene	24 hours
Carbons	TPC, TIC, TC	Cool to 4°C and keep out of sunlight. Transport to lab as soon as possible.	125 mL polyethylene	24 hours
Sulfide	Sulfide	Orange Dot (2 mL 1M Zinc Acetate)	300 mL glass BOD bottle	24 hours

nstructions:
. Assure that sample bottle is dust-free. Rinse with sample if possible.
. Break off ampoule top and empty into 3/4 filled sample bottle. CAUTION: Strong acids being handled.
. Fill bottle completely with sample.

OTE: Avoid using any intermediate sampling container which could contaminate the sample.

7.2 Fish

Dying fish should be collected for laboratory analysis of chemical residues (Table 1). Samples should be kept as cool as possible, preferably frozen until they arrive at the laboratory. The fish can generally be wrapped in washed aluminum foil regardless of the type of chemical involved in the kill. If aluminum foil is not available, plastic bags may also be useful if they are rinsed, but may impart some organic chemicals to the fish. Glass containers may also be used without releasing interfering substances. Do not add ice directly to the bag containing fish because of the possibility of contamination of the samples with ice water.

8.0 CONTINUITY

All samples must be handled in a way to eliminate the possibility of tampering. Each sample should be labelled as to date, time, location, sampling site, investigator. The sample should be sealed, either by a lock or with tape that can be initialled along the seams. All of the material is then locked in an appropriate tamper-proof container.

Ideally, the investigator would deliver the samples directly to the laboratory. If this is not possible, it is necessary to document the chain of custody of the samples. Each individual in the chain is required to sign for the receipt of materials. A minimum number of

people should be involved in the chain. The laboratory will also ensure continuity of samples during analysis.

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APPENDIX A. STANDARD PROCEDURE FOR PRESERVATION AND NECROPSY OF FISH

PROCEDURE: Necropsy of Fish

PRINCIPLE: To obtain well-fixed and uniform samples of fish tissue for histology.

EQUIPMENT AND REAGENTS:

1. Neutral buffered saline formalin
2. Bouin's fixative
3. Suitably sized tissue containers
4. Large pan scale for weighing the fish
5. Fish measuring board
6. Lens paper
7. Scalpel and disposable blades
8. Large scissors
9. Tissue forceps
10. Small curved scissors
11. Necropsy paper
12. Polyethylene bags for disposal of remains

SOLUTIONS:

1. Neutral Buffered Saline Formalin

Sodium phosphate monobasic	100 g
Sodium phosphate dibasic	162.5 g
Sodium chloride	2.25 g
Formaldehyde (37-40%)	2.5 L
Distilled water	23.5 L

OR

2. Bouin's Fixative

Picric acid, saturated aqueous	750 ml
Formaldehyde (37-40%)	250 ml
Glacial acetic acid	50 ml

Fix tissue from 4 to 24 hours depending on size. Wash tissue in 50% alcohol (several changes) to insure proper removal of the picric acid. Store trimmed tissue in 70% alcohol.

TECHNIQUE:

1. A suitably sized container is 2/3 filled with Bouin's fixative, into which all tissue samples are placed. Container is labelled with accession number and all other pertinent information.

2. The fish is received in the necropsy room together with an accession sheet containing its accession number.

3. The fish is weighed and a fork length measurement is taken, this information is recorded on the accession sheet. The external surface of the fish is examined for lesions and other abnormalities. If any abnormalities are found, a description is recorded on the accession sheet. Samples of any lesions will be taken for histology.

4. The fish is then placed on the necropsy table left side up.

5. Use a scalpel blade to remove a small piece of muscle tissue, 2 cm x 1 cm from behind the dorsal fin.

6. A square piece of skin is removed from the top of the head using a scalpel blade; this is placed on a piece of lens paper which is folded over it, and placed in fixative.

7. The eyes are removed by using small curved scissors and carefully cutting around the eye and behind it severing the optic nerve. The eye is then lifted out of the socket with forceps.

8. The operculum (on the left side) is cut away exposing the gills and gill arch. The top two gill sections are then removed and placed in the fixative.

9. The abdominal cavity is opened along the ventral midline from the anus to the lower jaw being careful not to cut into the internal organs.

10. The hypaxial musculature is cut away from the anal opening to the gill opening to meet the previous cut behind the lower jaw. This muscle flap is discarded.

11. As tissue samples are removed from the internal organs, each organ is examined for any gross abnormality. If abnormalities are found, a description is recorded on the accession sheet, and a sample of the affected tissue is placed in fixative.

12. The spleen is removed and an incomplete cut is made through the central area.

13. The heart is cut away with small curved scissors, being careful to remove the ventricle, atrium and the bulbous arteriosus. A small incision is made in the tip of the ventricle to insure proper fixation.

14. Cut the liver away, with or without the gall bladder. A 0.5-1 cm thick cross section of the liver is placed in the fixative.

15. The sex of the fish is determined by removing and examining the gonads. This information is recorded on the accession sheet. A cross section (up to 1 cm or if very small the whole organ) is taken for histology.

16. The remains of the gill arch are removed and discarded, and a portion of the esophagus (gullet) is taken for histology.

17. The remainder of the esophagus is opened longitudinally, the stomach. A cross section of a portion of the stomach is taken.

18. A cross section of the pyloric caecum and intestine is excised for histology.

19. The remnants of the internal viscera are removed, and a longitudinal incision is made on each side of the kidney. One portion of the kidney near the head is removed, and another sample of the kidney nearer to the tail is obtained.

20. The brain is removed by carefully opening the skull, prying back the bones and exposing the brain. The brain is carefully severed from the spinal cord and the major nerves by using small curved scissors to cut underneath; the brain is then lifted out. The brain may also be obtained by cutting the head transversely just posterior to the brain and lifting up the top of the skull.

21. A check is made of the organs required to be sure that they have all been collected and placed in fixative and that all necessary information has been recorded.

22. The remaining fish tissue is placed in a plastic bag for disposal.

APPENDIX B. STANDARD OPERATING PROCEDURE FOR RECEIPT OF EFFLUENTS FOR BIOASSAY TESTING

INTRODUCTION:

This document establishes standard operating procedures for the receipt and storage of liquid effluents (or samples) to be processed in fish bioassay tests.

PROCEUDRE:

1. During transportation and storage, the samples are kept in sealed polyethylene-lined containers, excluding all air.

2. The sample is not aerated or modified in any other fashion during storage.

3. The sample should not be stored for more than 5 days prior to commencing the bioassay.

4. The sample is stored at 4 ± 1°C.

5. When the effluent arrives at the Centre, it is logged into the appropriate data book and cross-referenced to the Acute Bioassay Master Book.

6. Upon receipt, the pH, dissolved oxygen, temperature and conductivity of the sample is measured. Ammonia is measured as required.

7. The sample is stored in a locked sample compartment in Cold Room B158.

8. A sample history sheet is set up and maintained (see attached).

REFERENCES

American Public Health Association. 1985. Standard methods for the examination of water and wastewater. 16th ed. American Public Health Association, Washington, D.C. 1268 p.

British Columbia Bioassay Task Force. 1982. Provincial guidelines and laboratory procedures for measuring acute lethal toxicity of liquid effluents to fish. Ministry of Environment, Waste Management Branch, Victoria, Canada. 18 p.

Environmental Protection Service, Environment Canada, 1980. Standard procedure for testing the acute lethality of liquid effluents. Supply and Services, Ottawa, Canada. 11 p.

McGuinness, E.J. 1982. Procedures manual; aquatic bioassay service projects. Alberta Environmental Centre, Vegreville, Canada. AECV-82M1. 56 p.

APPENDIX C. STANDARD OPERATING PROCEDURE - 96h STATIC BIOASSAY

INTRODUCTION:

This document establishes standard operating procedure for the performance of (a) regulatory, (b) research and (c) legal 96-h static bioassays. The procedures and conditions given below apply to the three categories of bioassays above except where noted. Amendments may exist for specific protocols, especially for research bioassays.

PROCEDURES:

A. Test conditions

1. Rainbow trout (Salmo gairdneri) are used as the test species.

2. Only healthy stocks of acclimated fish are used. Mortality of the fish lot for 4 days prior to the start of the bioassay should be less than 5%. Total mortality of the fish lot should be less than 10% (American Public Health Association, 1985). Fish should be actively feeding for a period of 2 weeks prior to the test.

3. Individual fish are to weigh between 0.5 and 10 g and the length of the largest fish should not exceed twice the length of the smallest fish.

4. The fish are not fed for 2 days prior to the bioassay or during the test.

5. Five concentrations of the bioassay sample plus a control are tested. The concentrations are 0, 20, 40, 60, 80 and 100% effluent. Recycled fish-holding water is used as the diluent.

6. Five to ten test fish per tank are exposed for 96 h.

7. For every one gram of fish there should be at least 0.5 L of test solution for every 24 h the fish are exposed.

8. The minimum solution depth in any test vessel should be 15 cm.

9. An aeration rate of 5-7.5 cm^3/min L should be applied to the test solution throughout the test period.

10. The test should be conducted at 15 ± 1°C.

11. Except for aeration, no food or chemicals are added to the test solutions.

12. The number of dead fish is observed at 0, 0.25 h, 0.5 h, 1 h, 2 h, 4 h, 24 h, 48 h, 72 h and 96 h. Dead fish are removed immediately.

13. For highly coloured or opaque samples, fish are inspected by raising them to the surface in a suitable basket or by carefully drawing a dip net through the test tank.

B. Test procedures:

1. Six polyethylene bags are rinsed 3 times with dilution water (treated municipal water). The bags are checked for holes. Each test tank is lined with a new polyethylene bag.

2. The diluent is metered into the test tanks using a G.P.I. digital flow meter (5 units = 20 L). The appropriate volumes are given in Table 1 based on either 20 or 40 L volumes of test solution.

3. On the Friday preceding the start of the bioassay, the test solutions are poured and thoroughly mixed for 2-5 min. The sample must be thoroughly mixed prior to metering out with G.P.I. flow meter and pump.

4. The bags are sealed and left over the weekend to acclimate to 15° C. The bioassay lab is locked during this time and access is restricted.

5. The bioassay is started at 0900 h on the Monday following set up of the test.

6. The pH, dissolved oxygen, temperature and conductivity (ammonia as required) of each test solution is measured at the start of the bioassay and at 24 h intervals for the duration of the test.

7. Mortality checks are carried out as per II.1.12. Depending upon the response of the fish, the frequency of checks may increase. Fish are considered dead when there are no respiratory or other movements and no response to gentle prodding.

8. Dead fish are removed as per II.1.12 and their fork lengths and wet weights recorded.

9. At the termination of the bioassay, all remaining fish are killed as per SOP 2350-AJ4-1/AC.2.

Table 1. Effluent Dilutions for Twenty and Forty Litre Bioassay Tests

Total Test Volume Required (L)	Desired Concentration (%)	Volume of Dilution Water (L)	Volume of Effluent (L)
20	100	0	20
	80	4	16
	60	8	12
	40	12	8
	20	16	4
	Control	20	0
40	100	0	40
	80	8	32
	60	16	24
	40	24	16
	20	32	8
	Control	40	0

10. All data is recorded on the appropriate data sheets (see attached) and in the appropriate log books.

11. The data are analysed following standard procedures.

C. Interpretation of Test Results:

1. The test is considered invalid if mortality exceeds 10% in the control tank.

2. An effluent passes the standard test for acute lethality if more than 50% of the fish survive 96 h exposure to 100% effluent under the standard conditions. An effluent fails the test if 50% or less of the test fish survive 96 h exposure to 100% effluent under the standard conditions.

This procedure requires that an effluent sample not be diluted prior to the assay, however some effluent regulations and/or guidelines describe dilution allowances, and they should be referred to prior to commencing the test (Environment Protection Service, 1980).

REFERENCES

American Public Health Association. 1985. Standard methods for the examination of water and wastewater. 16th ed. American Public Health Association, Washington, D.C. 1268 p.

British Columbia Bioassay Task Force. 1982. Provincial guidelines and laboratory procedures for measuring acute lethal toxicity of liquid effluents to fish. Ministry of Environment, Waste Management Branch, Victoria, Canada. 18 p.

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McGuinness, E.J. 1982. Procedures manual aquatic bioassay service projects. Alberta Environmental Centre, Vegreville, Canada. AECV-82M1. 56 p.

APPENDIX D. STANDARD OPERATING PROCEDURE FOR CHEMICAL REFERENCE TOXICANT BIOASSAY

INTRODUCTION:

This document establishes standard operating procedure for the performance of a chemical reference toxicant bioassay. The purpose of such bioassays is to quantify the toxicant sensitivity of a particular fish lot. Phenol is a systemic toxicant that affects many physiological functions in fish and, for that reason, has been chosen as the reference toxicant.

PROCEDURE:

A. Test Conditions:

1. The test conditions meet those listed in SOP 2350-AJ4-1/Lab. Gen.4 96 h static except where deviations are specifically noted.

2. The duration of the bioassay is 24 h.

3. The concentrations tested are 0, 5, 8, 10, 12 and 15 mg/L phenol.

B. Test Procedures:

1. The test procedures follow that of SOP 2350-AJ4-1/Lab. Gen.4 except as noted.

2. The phenol stock solution is prepared the day of the test (Table 1).

3. The pH, dissolved oxygen, temperature and conductivity of each toxicant solution are measured at the start of the test and after 24 h.

4. The bioassay results are analysed by standard procedures and logged with the appropriate 96 h LC_{50} test results.

5. A chemical reference summary sheet is set up and maintained (see attached).

C. Interpretation of Test Results:

1. The normal 24 h LC_{50} for rainbow trout (Salmo gairdneri) occurs between 8 and 12 mg/L phenol.

Table 1. Phenol Stock Solution and Dilutions

Volume of stock solution required (ml) =

$$\frac{1000 \text{ ml [desired concentration (mg/L)]}}{\text{stock concentration (mg/L)}}$$

A 3000 mg/L stock solution is prepared.

Desired Concentration (mg/L) ml stock/40 L	ml Stock/20L
66.67	33.33
106.67	53.33
133.33	66.67
160.00	80.00
200.00	100.00

REFERENCES

American Public Health Association. 1985. Standard Methods for the examination of water and wastewater. 16th ed. American Public Health Association, Washington, D.C. 1268 p.

British Columbia Bioassay Task Force. 1982. Provincial guidelines and laboratory procedures for measuring acute lethal toxicity of liquid effluents to fish. Environment, Waste Management Branch, Victoria, Canada. 18 p.

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